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C6F FAP F101
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U1S S1289 S1336

(56) Documents Cited
WO 90/02167 A1 **US 4668633 A**

(58) Field of Search
UK CL (Edition N) **C6F FAP**
INT CL^6 **C12M 1/00 1/04 1/10 1/16 1/22 1/24**
ONLINE: WPI,CLAIMS

(54) **Bacterial culture vessel having a lid able to hold encapsulated culture supplements**

(57) A bacterial culture vessel is made of plastics to be economically disposable. It has a container ② with internal fins ⑦ and a snap-on lid ③ . The container ② and lid ③ are packaged separately in a sterile manner, and the container can be prepacked with dehydrated culture medium ⑧ . Conveniently it will stack with other packaged containers. Part of the lid is a blister-pack containing supplements ⑤ . With the lid in place, these can be selectively released into the container by locally pressing the top of the lid ③ . The lid also has a closable aperture ④ for introducing bacterial inoculum and for aeration. The culture vessel can also adapt, via screw-thread ⑨ , into a base ① , allowing it to be held ⑥ onto a moving platform whilst culturing is in progress.

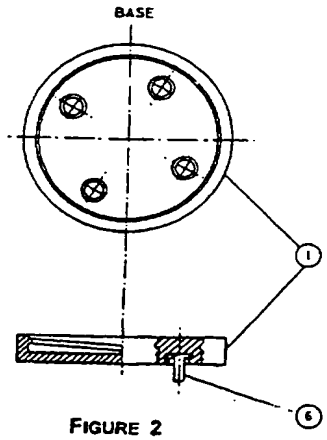

FIGURE 2

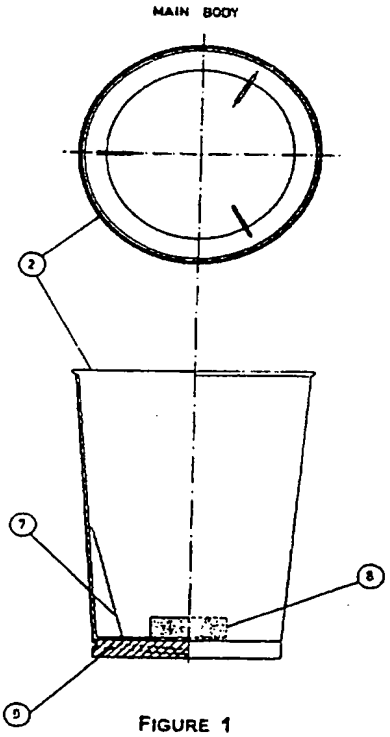

FIGURE 1

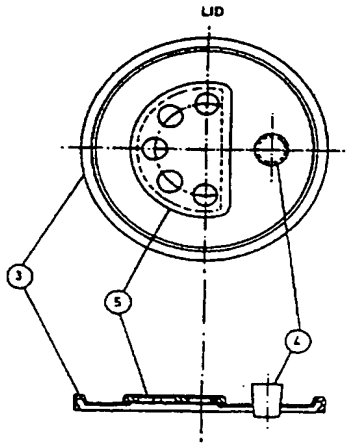

FIGURE 3

DISPOSABLE CULTURE VESSEL

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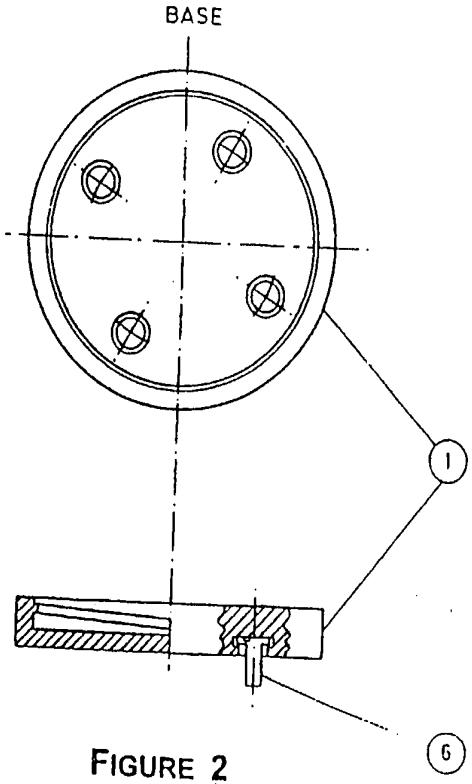

FIGURE 2

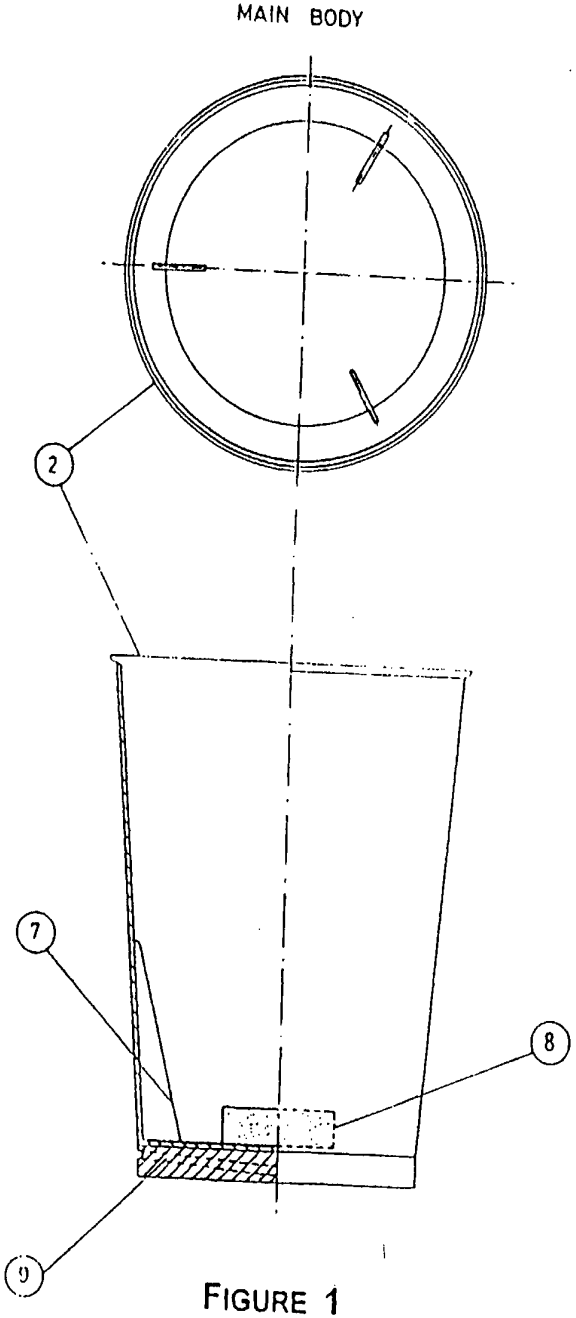

FIGURE 1

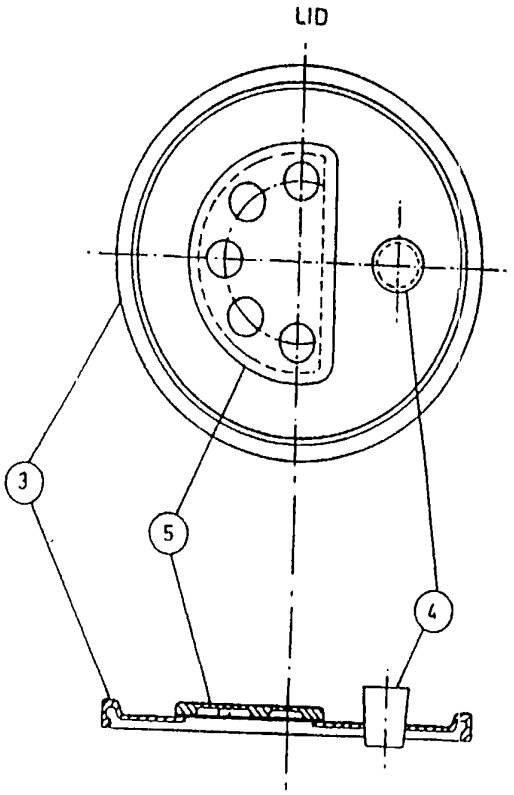

FIGURE 3

DISPOSABLE CULTURE VESSEL

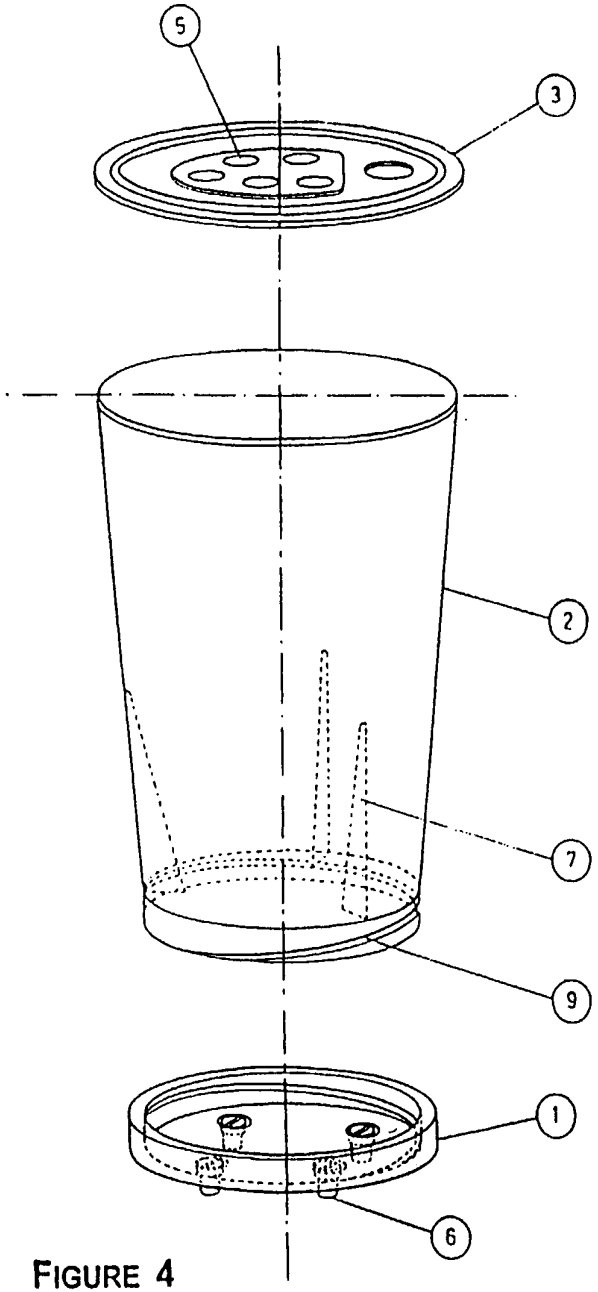

FIGURE 4

STACKABLE CULTURE VESSELS

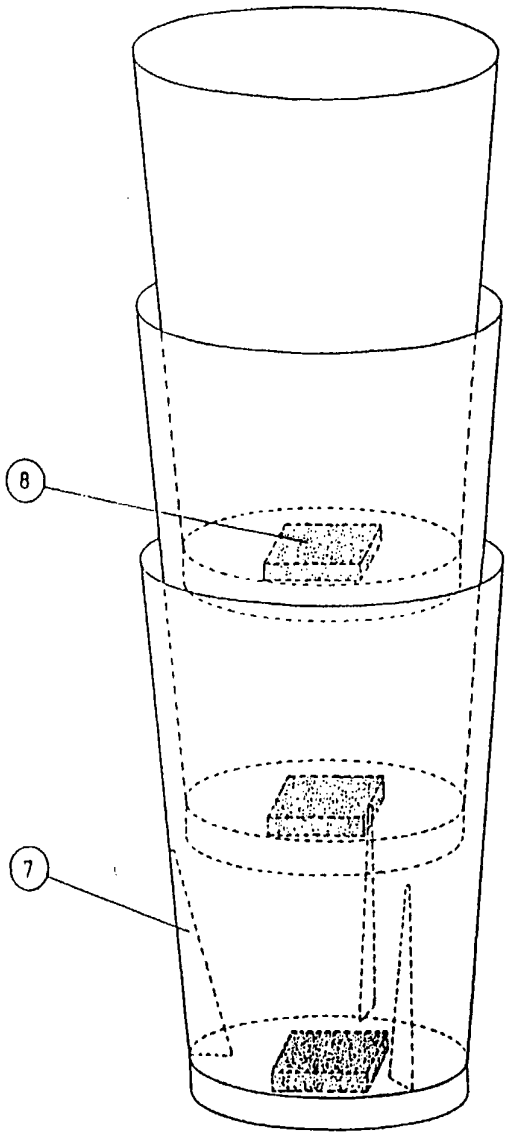

FIGURE 5

IMPROVEMENTS RELATING TO BACTERIAL CULTURE VESSELS

This invention relates to the scientific procedure of bacterial cell culture.

The culture of bacteria in laboratories is a necessary prerequisite for both medical and biotechnological research in the animal and plant fields. Bacterial culture is presently carried out in sterile glassware. To set up a bacterial growth culture, a sterile glass flask containing liquid growth medium is inoculated with a bacterial sample. When the culture is placed at 37°C, it will support the vigorous growth of the bacterial inoculum in the laboratory. The rapid growth of a bacterial culture following these procedures is common, and is currently widely used in molecular biology research laboratories world-wide.

Currently, to prepare suitable sterile liquid growth medium, before a bacterial inoculum can be added, precise quantities of different powder culture media must be weighed out accurately and added to a specific volume of distilled water in glass flasks. The liquid culture medium in the glassware must then be sterilised (by autoclaving) and cooled prior to use. Only then, may the bacterial inoculum be added to the culture medium along with the necessary supplements (antibiotics). The bacteria inoculated culture is then placed on an orbitally rotating platform in a 37°C incubator; conditions that allow for extensive bacterial growth.

As mentioned, bacterial culture using this procedure is widely used in research laboratories. However, the technique has many disadvantages for:

1. It requires the use of fragile glassware. Glassware is costly, expensive to maintain (requiring washing and sterilising) and requires a lot of storage space. Glassware is also fragile which makes replacement imminent.

2. The preparation of sterile bacterial growth media is an elaborate and time-consuming procedure. The preparation of sterile liquid growth media requires accurate weighing of media and measurement of distilled water, factors which are also subject to human error. The sterilisation steps, which make use of an autoclave, take about two hours. Together, these preparation steps substantially increase the necessary time required to complete an experiment. Furthermore, detergent residues are often left deposited on washed glassware which inhibits the growth of bacteria in new experiments. This adds considerable financial and temporal cost by ruining these experiments.

The aim of this invention is to replace the existing antiquated procedure and simplify the process.

According to one aspect of the present invention there is provided a bacterial culture vessel comprising an open-topped receptacle with a lid, the lid having means to hold encapsulated supplement for the culture medium and a closable aperture for aeration and for the introduction of bacterial inoculum into the receptacle, the lid being manipulable when in place on the receptacle to break the underside of the encapsulation and release the supplement into the culture medium.

Conveniently, the lid is at least partially of blister pack construction with the supplement in a blister and contained by a foil over the underside of the blisters which is breakable by the manipulation. Generally there will be a plurality of such blisters each with a supplement allowing selective additions to the culture medium.

The aperture may also be initially closed by a foil on the underside of the lid, and there would be a gas permeable bung to close it during the culturing process.

Preferably, the receptacle will be of beaker-like form with internal fins to assist turbulence of the liquid contents when shaken. These fins can also serve as mechanical strengtheners. With the receptacle of moulded plastics construction, this means that the plastics can be quite thin.

The receptacle may have its lower end adapted for positive retention on a moving base. For example, it may be screw threaded to engage a complementary screw threaded socket on a turntable.

The lid, which will also be of plastics material, will preferable be a snap fit on to the receptacle.

The receptacle can be sterilely packaged separately from the lid, and dehydrated culture medium may be provided in the receptacle within its package. Conveniently, the packaged receptacle can nest with a similar one so that a plurality of them can be temporarily stacked.

According to another aspect of the present invention there is provided a method of culturing bacteria, wherein such a culture vessel as outlined above and containing dehydrated culture medium is charged with water, the lid is fitted, one or more selected supplements are released into the liquid medium by lid manipulation, the vessel is agitated, bacteria is inoculated into the liquid medium via said aperture, and the aperture is closed for culturing to proceed.

The introduction of stackable, plastic disposable bacterial culture vessels for bacterial cell growth could *eliminate* or at least substantially reduce the problems associated with glassware and substantially increase the efficiency and rapidity of the time it takes to prepare suitable culture media (with disposable plasticware, it will take only 15 minutes (or $^1/_8$ of the time) to prepare suitable growth media). It could improve the reliability and performance of this important procedure used widely in many scientific institutions.

For a better understanding of the invention, one example will now be described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows a vertical section through a bacterial culture vessel, and a view looking directly from above.

Figure 2 shows a vertical section through a screw base, and a view looking directly from above.

Figure 3 shows a vertical section through the middle of a lid for the vessel, and a view looking directly from above.

Figure 4 shows a bacterial culture vessel with its screw base and lid, seen in perspective, partially from above.

Figure 5 illustrates in perspective that bacterial culture vessels are stackable.

The disposable culture vessel is a sterile, translucent, cylindrical plastic container ②. The vessels have a range of liquid capacities (50, 100, 500, 1000 ml volumes - the number specifying the volume of liquid added) to support different size cultures.

Each vessel contains internally moulded aeration fins ⑦, and the base of each vessel has a screw-fitting with a quick-turn thread ⑨. A cube (tablet) of pre-weighed powdered bacteria culture medium ,⑧, is supplied pre-packaged inside each vessel. The specific composition of the powdered medium cube can vary depending on user requirement i.e., similar culture vessels can be supplied with media of different composition depending on the user's needs.

The fins provide two distinct functions:
(i) When the liquid culture medium is shaken on the rotating platform in the incubator, the fins increase the turbulence in the liquid, and in so doing, provide needed extra aeration to the culture.
(ii) Internally moulded fins add substantial rigidity and strength to the plastic vessel making possible the use of thinner and therefore cheaper plastic for the rest of the container.

The culture vessels seal at the top with a snap on lid, ③, which is provided with each vessel but are packaged separately. The lid is circular and incorporates a blister-pack. The underside of each lid is covered by a sheet of aluminium foil which holds the contents of the 'blisters' in place. The edges of the blister pack have a flange which allows them to snap onto the top of each culture vessel to close them.
Each blister pack is pre-packaged with a range of different antibiotic tablets (medium supplements). These may be dispensed by pressing any of the blisters on the lid of the blister pack (⑤).
An aeration aperture is also present in each lid for the addition of the bacterial inoculum. This plugs with a gas-permeable stopper ④. Initially, it may also be spanned by aluminium foil which is pierced when the bacterial inoculum is added, and then stoppered.

The screw-thread at the bottom of each vessel fits and screws into a separate Screw base ①. This screw base is permanently attached to the rotating platform of a standard 37°C incubator (as previously described). The screw base will be supplied separately as a non-recurring purchase and will serve to adapt all culture vessels to all current orbital shakers.

The culture vessels are also stackable. This reduces their bulk which will ease their transport and reduce the valuable laboratory space required for their storage. The stackable vessels along with lids will be supplied sterile (by previous irradiation) in plastic packing sleeves. The packing sleeves will ensure that the individual culture vessels and lids remain sterile. Boxes containing sterile stackable culture vessels in packing sleeves, along with lids, can also be shipped, delivered, and stored in laboratories in this manner.

To make use of the stackable, disposable bacterial culture vessel, only the following steps will be needed:

1. Removal of a single culture vessel ① and a lid ③ from the packing sleeves under aseptic conditions.

2. Filling of the culture vessel with filtered (sterile) water up to the level of the internally moulded aerating fins ⑦. Closing of the vessel by snapping on the lid.

3. Dispensing the appropriate medium supplements (antibiotics) which are supplied in the lid as blister-pack tablets.

4. Swirling the box to dissolve the contents; then inoculate the liquid medium with bacteria via the aeration aperture ④ which can then be stoppered with a foam rubber plug.

5. Screwing the entire culture-containing culture vessel into the base ①, which is pre-attached (by screws) to the orbitally rotating platform in the 37°C incubator.

6. Culturing as normal.

7. After culture, unscrewing the culture vessel from its screw base on the rotating shaker in the 37°C incubator. The resulting bacteria broth can then be used, whilst the plastic culture vessel is disposed.

By supplying the culture vessels complete with dried medium and supplements, the requirement for separate procedures for making, dispensing and sterilising culture media is bypassed, thus offering the user a substantial time-advantage and assured medium quality. Only a simple filtered water supply is needed, thus making it the most convenient approach to bacterial culture, a feature which will also be most appreciated by the occasional user not having ready access to all the necessary reagents, facilities for high-volume autoclaving, and glassware storage space.

CLAIMS

1. A bacterial culture vessel comprising an open-topped receptacle with a lid, the lid having means to hold encapsulated supplement for the culture medium and a closable aperture for aeration and for the introduction of bacterial inoculum into the receptacle, the lid being manipulable when in place on the receptacle to break the underside of the encapsulation and release the supplement into the culture medium.

2. A culture vessel as claimed in Claim 1, wherein the lid is at least partially of blister pack construction with the supplement in a blister and contained by a foil over the underside of the blister which is breakable by the manipulation.

3. A culture vessel as claimed in Claim 2, wherein there is a plurality of such blisters each with a supplement allowing selective additions to the culture medium.

4. A culture vessel as claimed in any preceding claim, wherein the aperture is initially closed by a foil on the underside of the lid.

5. A culture vessel as claimed in any preceding claim, and including a gas-permeable bung for the aperture.

6. A culture vessel as claimed in any preceding claim, wherein the receptacle is of beaker-like form with internal fins to assist turbulence of liquid contents when shaken.

7. A culture vessel as claimed in any preceding claim, wherein the receptacle is of moulded plastics construction.

8. A culture vessel as claimed in any preceding claim, wherein the receptacle has its lower end adapted for positive retention on a moving base.

9. A culture vessel as claimed in any preceding claim, wherein the lower end of the receptacle is screw threaded.

10. A culture vessel as claimed in any preceding claim, wherein the lid is a snap fit onto the receptacle.

11. A culture vessel as claimed in any preceding claim, wherein the receptacle is sterilely packaged separately from the lid.

12. A culture vessel as claimed in Claim 11, wherein dehydrated culture medium is provided in the receptacle within its package.

13. A culture vessel as claimed in Claim 11 or 12, wherein the packaged receptacle can nest with a similar packaged receptacle, so that a plurality of such packaged receptacles can be compactly stacked.

14. A bacterial culture vessel substantially as herein before described with reference to the accompanying drawings.

15. A method of culturing bacteria, wherein a culture vessel as claimed in any preceding claim and containing dehydrated culture medium is charged with water, the lid is fitted, one or more selected supplements are released into the liquid medium by lid manipulation, the vessel is agitated, bacteria is inoculated into the liquid medium via said aperture, and the aperture is closed for culturing to proceed.

Amendments to the claims have been filed as follows

1. A bacterial culture vessel comprising an open-topped receptacle with a lid, the lid having means to hold encapsulated supplement for the culture medium and a closable aperture for aeration and for the introduction of bacterial inoculum into the receptacle, the lid being manipulable when in place on the receptacle to break the underside of the encapsulation and release the supplement into the culture medium.

2. A culture vessel as claimed in Claim 1, wherein the lid is at least partially of blister pack construction with the supplement in a blister and contained by a foil over the underside of the blister which is breakable by the manipulation.

3. A culture vessel as claimed in Claim 2, wherein there is a plurality of such blisters each with a supplement allowing selective additions to the culture medium.

4. A culture vessel as claimed in any preceding claim, wherein the aperture is initially closed by a foil on the underside of the lid.

5. A culture vessel as claimed in any preceding claim, and including a gas-permeable bung for the aperture.

6. A culture vessel as claimed in any preceding claim, wherein the receptacle is of beaker-like form with internal fins to assist turbulence of liquid contents when shaken.

7. A culture vessel as claimed in any preceding claim, wherein the receptacle is of moulded plastics construction.

8. A culture vessel as claimed in any preceding claim, wherein the receptacle has its lower end adapted for positive retention on a moving base.

9. A culture vessel as claimed in any preceding claim, wherein the lower end of the receptacle is screw threaded.

10. A culture vessel as claimed in any preceding claim, wherein the lid is a snap fit onto the receptacle.

11. A culture vessel as claimed in any preceding claim, wherein the receptacle is sterilely packaged separately from the lid.

12. A culture vessel as claimed in Claim 11, wherein dehydrated culture medium is provided in the receptacle within its package.

13. A culture vessel as claimed in Claim 11 or 12, wherein the packaged receptacle can nest with a similar packaged receptacle, so that a plurality of such packaged receptacles can be compactly stacked.

14. A bacterial culture vessel substantially as herein before described with reference to the accompanying drawings.

15. A method of culturing bacteria, wherein a culture vessel as claimed in any preceding claim and containing dehydrated culture medium is charged with water, the lid is fitted, one or more selected supplements are released into the liquid medium by lid manipulation, the vessel is agitated, bacteria is inoculated into the liquid medium via said aperture, and the aperture is closed for culturing to proceed.

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Patents Act 1977 **Examiner's report to the Comptroller under Section 17** **(The Search report)**	Application number GB 95[illegible]95.6
Relevant Technical Fields (i) UK Cl (Ed.N) C6F (FAP) (ii) Int Cl (Ed.6) C12M 1/00, 1/04, 1/10, 1/22, 1/24, 1/16	Search Examiner MR C SHERRINGTON
	Date of completion of Search 4 DECEMBER 1995
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE: WPI, CLAIMS	Documents considered relevant following a search in respect of Claims :- 1 TO 15

Categories of documents

X:	Document indicating lack of novelty or of inventive step.	P:	Document published on or after the declared priority date but before the filing date of the present application.
Y:	Document indicating lack of inventive step if combined with one or more other documents of the same category.	E:	Patent document published on or after, but with priority date earlier than, the filing date of the present application.
A:	Document indicating technological background and/or state of the art.	&:	Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
A	WO 90/02167 A1	(ANDIRAL S.A.L) whole document	1
A	US 4668633	(JOHN R WALTON) especially Figures 2 and 3; column 5, line 58 to column 6, line 9	1

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).